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(54) METHOD OF INDUCING AND MAINTAINING NEURONAL CELLS

(54) PROCEDE D'INDUCTION ET DE MAINTIEN DE CELLULES NEURONALES

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ABSTRACT:

The present invention makes available a method for inducing neuronal differentiation and preventing the death or degeneration of neuronal cells both in vitro and in vivo. The subject method stems from the unexpected finding that, contrary to traditional unterstanding of neural induction, the default fate of ectodermal tissue is neuronal rather than mesodermal and/or epidermal. In particular, it has been discovered that preventing or antagonizing a signaling pathway in a cell for a growth factor of the TGF-.beta. family can result in neuronal differentiation of that cell.

CLAIMS: Show all claims

*** Note: Data on abstracts and claims is shown in the official language in which it was submitted.

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Patent Document Number 2174098 :
METHOD OF INDUCING AND MAINTAINING NEURONAL CELLS

PROCEDE D'INDUCTION ET DE MAINTIEN DE CELLULES NEURONALES

CLAIMS:

CLAIMS:

1. A method for inducing a cell to differentiate to a neuronal cell phenotype, comprising contacting said cell with an agent which antagonizes the biological action of at least one polypeptide growth factor of the Transforming Growth Factor-.beta. (TGF-.beta.) family, said growth factor normally inducing said cell to differentiate to a non-neuronal phenotype.

2. The method of claim 1, wherein said antagonizing agent inhibits the biological activity of said growth factor by preventing said growth factor from binding growth factor receptors on the surface of said cell.

3. The method of claim 2, wherein said antagonizing agent binds said growth factor and sequesters said growth factor such that it cannot bind said growth factor receptors.

4. The method of claim 3, wherein said antagonizing agent is selected from a group consisting of a follistatin, an .alpha.2-macroglobulin, a protein containing at least one follistatin module, and a truncated receptor for a growth factor of the TGF-.beta. family.

5. The method of claim 4, wherein said truncated receptor comprises a soluble growth factor-binding domain of a TGF-.beta. receptor.

6. The method of claim 5, wherein said truncated receptor comprises a truncated activin receptor.

7. The method of claim 2, wherein said antagonizing agent inhibits binding of said growth factor with said growth factor

receptors via its own binding to said growth factor receptor.

8. The method of claim 7, wherein said antagonizing agent is an inhibin.

9. The method of claim 7, wherein said antagonizing agent is a polypeptide of said TGF-.beta. family and which has one or more sites of amino acid mutation, said mutation diminishing an ability of said TGF-.beta. polypeptide to induce said cell to differentiate to a non-neuronal phenotype, yet not substantially diminishing the binding of said activin to said growth factor receptor.

10. The method of claim 9, wherein said TGF-.beta. polypeptide is a mutated activin.

11. The method of claim 7, wherein said antagonizing agent is peptidyl fragment, or a peptidomimetic thereof, of a receptor-binding portion of an activin or inhibin protein.

12. The method of claim 1, wherein said antagonizing agent is an antisense nucleic acid construct which inhibits expression of a receptor for said TGF-.beta. polypeptide.

13. The method of claim 1, wherein said antagonizing agent is dominant negative TGF-.beta. receptor comprising an extracellular growth factor-binding domain of a TGF-.beta. receptor, a transmembrane domain for anchoring said extracellular domain to a cell surfacemembrane, and a dysfunctional cytoplasmic domain, said dominant negative receptor being recombinantly expressed in said cell and inhibits the biological activity of said growth factor by inhibiting signal transduction by a naturally-occurring TGF-.beta. receptor.

14. The method of claim 1, wherein said growth factor is activin.

15. The method of claim 1, wherein said cell is further contacted with a second growth factor having neurotrophic or neural inductive activity, such as a nerve growth factor, cilliary neurotrophic growth factor, schwanoma-derived growth factor, glial growth factor, stiatal-derived neuronotrophic factor, platelet-derived growth factor, scatter factor, a vertebrate hedgehog protein, noggin, and a ligand for a Notch receptor.

16. The method of claim 1, wherein said cell is part of a host organism, and said antagonistic agent is delivered in the form of an in vivo therapeutic formulation.

17. The method of claim 1, wherein said neuronal cell comprises a neural progenitor cell.

18. The method of claim 1, wherein said neuronal cell is selected from a group consisting of a melanocyte progenitor cell, a glial progenitor cell, a sensory neuron progenitor cell, a sympatho-adrenal progenitor cell, a parasympathetic progenitor cell, and an enteric progenitor cell.

19. The method of claim 1, wherein said neuronal cell is a terminally-differentiated
neuronal cell.

20. The method of claim 19, wherein said terminally-differentiated neuronal cell is selected from a group consisting of a microglial cell, a macroglial cell, a schwann cell, a cholinergic cell, a peptidergic cell, and a serotenergic cell.

21. The method of claim 1, wherein said cell is selected from a group consisting of an
embryonic cell, a fetal cell, and a neonatal cell.

22. A method for preventing death of a neuronal cell comprising contacting said cell with an agent which antagonizes the biological action of at least one polypeptide growth factor of the Transforming Growth Factor-.beta. (TGF-.beta.) family, said growth factor normally inducing said cell to differentiate to a non-neuronal phenotype.

23. The method of claim 22, wherein said antagonizing agent is selected from a group consisting of a follistatin, a truncated activin receptor, an .alpha.2-macroglobulin, an inhibin, and an antagonistic mutant of a polypeptide growth factor of the TGF-.beta. family.

24. The method of claim 22, wherein said cell is further contacted with a second growth factor having neurotrophic activity, such as a nerve growth factor, cilliary neurotrophic growth factor, schwanoma-derived growth factor, glial growth factor, stiatal-derived neuronotrophic factor, platelet-derived growth factor, scatter factor, a vertebrate hedgehog protein, noggin, and a ligand for a Notch receptor.

25. A method for inducing a cell to differentiate along a determined neuronal pathway comprising, contacting said cell with an agent which disrupts a signaling pathway in said said cell of a growth factor of the TGF-.beta. family, said signaling pathway normally inducing said cell to differentiate to a non-neuronal cell-type.

26. The method of claim 25, wherein said signaling pathway is an activin-signaling
pathway.

27. A method for treating a degenerative disorder of the nervous system characterized by neuronal cell death, comprising administering to a patient a therapeutically effective amount of an agent which antagonizes the biological action of at least one polypeptide growth factor of the Transforming Growth Factor-.beta. (TGF-.beta.) family, said growth factor normally inducing cells in said patient to differentiate to a non-neuronal phenotype.

28. The method of claim 27, wherein said antagonizing agent is selected from a group consisting of a follistatin, a truncated activin receptor, an .alpha.2-macroglobulin, an inhibin, and an antagonistic mutant of a polypeptide growth factor of the TGF-.beta. family.

29. The method of claim 27, wherein said therapeutically effective amount of said antagonizing agent inhibits the de-differentiation of neuronal cells of said patient.

30. The method of claim 27, wherein said therapeutically effective amount of said antagonizing agent induces the terminal differentiation of cells of said patient to a neural cell phenotype.

31. The method of claim 30, wherein said neural cell phenotype is a glial cell.

32. The method of claim 30 wherein said neural cell phenotype is a nerve cell.

33. The method of claim 27 wherein said degenerative disorder is a neuromuscular disorder.

34. The method of claim 27, wherein said degenerative disorder is a autonomic disorder.

35. The method of claim 27, wherein said degenerative disorder is a central nervous system
disorder.

36. The method of claim 27, wherein said degenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Pick's disease, Huntington's disease, multiple sclerosis, neuronal damage resulting from anoxia-ischemia, neuronal damage resulting from trauma, and neuronal degeneration associated with a natural aging process.

37. The method of claim 27, wherein a therapeutically effective amount of a second growth
factor having neurotrophic activity is administered to said patient.

38. The method of claim 37, wherein said second growth factor is selected from a group consisting of a nerve growth factor, cilliary neurotrophic growth factor, schwanoma-derived growth factor, glial growth factor, stiatal-derived neuronotrophic factor, platelet-derived growth factor.

39. The method of claim 27, wherein a therapeutically effective amount of an antimitotic agent is administered to said patient in order to reduce the rate of growth of glial cells and favor the growth of nerve cells.

40. The method of claim 39, wherein said antimitotic agent is selected from a group consisting of cytosine, arabinoside, 5-fluorouracil, hydrozyurea, and methotrexate.

41. A method for identifying a neuralizing activity, comprising (i) culturing animal cap cells derived from an embryo, or equivalent cells thereof, in the presence of a polypeptide growth factor of the TGF-.beta. family, said growth factor normally inducing said cells to differentiate to a non-neuronal phenotype, (ii) contacting said cells with a candidate agent, and (iii) detecting the neuronal differentiation of any of said cells, wherein neuronal differentiation of said cells in the presence of said candidate agent is indicative of a neuralizing activity.

42. The method of claim 41, wherein said growth factor is activin.

43. The method of claim 41, wherein said neuronal differentiation is detected by scoring for the presence of a neural-specific marker on the surface of said cells.

44. The method of claim 43, wherein said neural specific marker is NCAM, and thepresence of NCAM is scored using a detectably labeled anti-NCAM antibody.

CELL AND MOLECULAR BIOLOGY

International Conference
Bone Morphogenetic Proteins 2000
June 7-11, 2000 - Granlibakken, Lake Tahoe, California

Abstract Form

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TYPE ABSTRACT HERE - YOU MUST STAY WITHIN BORDER

Noggin: Moleculer Developmental Biology

Vertebrate embryos employ a surprising number of mechanisms to suppress signalin BMPs. One such mechanism is the production of secreted BMP antagonists. We ha expression cloning in Xenopus embryos to isolate a variety of signaling molecules. embryo is exquisitely sensitive to manipulations that affect BMP signaling, since B important dose-dependent regulators of embryonic pattern. The assay has identified number of BMP antagonists, including *noggin* and *gremlin*. These proteins bind wit affinity to BMPs and thereby prevent BMPs' access to their cell surface receptors. T signaling properties of these antagonists in processes such as neural induction and m patterning illustrate the principle that blocking a signal can provide specific informat embryonic development. The antagonists are also used to regulate BMP signaling in tube patterning, somite differentiation, and other downstream events. Given the piv of BMPs in skeletal development, it should not be a surprise that BMP blocking sign as noggin serve to regulate cartilage condensation and joint development.

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The role of GDF-5 during skeletal development

P.H. Francis-West[1], A. Abdelfattah[1], P.Chen[2], F.P. Luyten[2,3] and C.W. Archer[4].

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Mutation of GDF-5 in mice and humans results in defects in the appendicular skeleton characterise a shortening of the skeletal elements in a proximal-distal gradient and abnormalities or loss of some joints. *Gdf-5* is first expressed in the cartilage condensations and later becomes localised to the join interzone. To understand the mechanisms of GDF-5 action we took gain-of-function approaches *in* and *in vivo* using the developing chick embryo as an experimental model. Misexpression of GDF-5 *vivo* using the replication competent retrovirus, RCAS(BP), resulted in an increase in the width an length of the skeletal elements. This was due to an increase in chondrocyte number and the effect w first apparent during early chondrogenesis. Micromass and cell suspension cultures showed that G can promote initiation of chondrogenesis by increasing cell adhesion in a subpopulation of cells. Misexpression of GDF-5 later during skeletal development also increased the size of the skeletal elements. Pulse labelling experiments showed that this was due, in part, to an increase in chondroc proliferation. Thus, GDF-5 appears to act by two distinct mechanisms in a stage-dependent manner.

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TYPE ABSTRACT HERE - YOU MUST STAY WITHIN BORDER

NOGGIN, A BMP ANTAGONIST, INCREASES PROLIFERATION, DIFFERENTIATION AND MINERALISATION IN HUMAN MARROW MESENCHYMAL STEM CELLS.

v.l. Church, S. Kenenedy, U. Patel, B.A. Ashton, Leopold Muller Arthritis Research Centre, Robert Jones & Agnes Hu Orthopaedic Hospital, Oswestry, Shropshire SY10 7AG

The role of BMPs in the proliferation and differentiation of osteoblasts from human marrow stromal stem c (HMMSC) has been explored with the use of the BMP antagonist, noggin.
The effect of noggin (a gift from Regeneron Pharmaceuticals, Inc., Tarrytown, NY) on fibroblast colony formation by human MMSC was established in limiting dilution assays. In three separate experiments, the colony forming efficiencies in the presence or absence of noggin did not differ significantly. In each experi however, colonies grown in the presence of noggin contained more cells than those in control medium ($p<0$ Mann-Whitney U-test).
When cultured with dexamethasone in combination with ascorbate-2-P and β-glycerophosphate, HMMSC differentiate into osteoblasts. Peak alk Pase activities, up to 30 times higher than control, were seen after 1 days. Thereafter calcium phosphate deposition in the extracellular matrix increased rapidly. The effects of noggin (1-100 ng/ml) on osteogenesis were entirely unexpected, in each of four experiments there was a significant dose related increase in both alkaline phosphatase activity (mean increase 56%, range 23-87%) a deposition (mean increase 132%, range 77-182%). Noggin was unable to replace for the need for dexamethasone in this in vitro model of osteogenesis.
As there is no evidence of noggin having a physiological role other than as a BMP antagonist, the results su that one (or more) of the BMPs antagonised by noggin inhibits the proliferation of marrow stromal cells an differentiation to osteoblasts.

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SITE AND TISSUE SPECIFICITY OF ENDOCHONDRAL BONE FORMATION BY TRANSFORMING GROWTH FACTOR-BETAS (TGF-βS) IN THE PRIMATE *Papio ursin*
Jacqueline Tasker, Thato Matsaba, Jean Crooks, June Teare and Ugo Rjpamonti, Bone Research U Johannesburg, South Africa.

The BMP members of the transforming growth factor-beta (TGF-β) superfamily induce bone forma *in vivo* when implanted in extraskeletal heterotopic sites of rodents. In such assays in rodents, TGF- are known to induce granulation tissue only, with occasionally marked fibrosis. However recombin human (h) TGF-β1 and -β2 have recently been found to be inducers of bone formation in the primat *Papio ursinus*, thus extending this function to other members of this superfamily. We have found th hTGF-β1 and -β2 induce endochondral bone formation 30 days after implantation in heterotopic intramuscular sites of the baboon *(Papio ursinus)* at doses of 1, 5 and 25 μg per 100 mg guanidiniu inactivated collagenous bone matrix (ICBM) as carrier. On day 90 there was generation of large corticalised intramuscular ossicles. The bone inductive activity of these two TGF-β isoforms is site tissue specific since neither induced bone in calvarial defects except to a very limited extent, pericranially, whilst both have been found to be inducers of endochondral bone differentiation in